THE MICROBIOLOGY OF A PODZOL PROFILE

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I have chosen to discuss the microbiology of a podzol profile for several reasons, but the two main ones are that it gives an opportunity to deal with a number of fundamental problems in soil microbiology and to demonstrate the value of using a wide range of techniques in one investigation.

In this paper I have drawn freely on the unpublished work of my colleagues and students over the last ten years. Some, like Drs. Parkinson, Williams and Gams, are presenting certain aspects of their work in detail but I shall also be making considerable use of results obtained by Dr. Kendrick an Mr. Nicholas.

The area chosen for study was a forest of *Pinus sylvestris* growing on glacial sands at Delamere, Cheshire. Historical records suggest that *Calluna* heath covered the area until a little over 100 years ago when pines were planted, natural regeneration has followed and the present trees are about 60—80 years old. The porous sands have been podzolized to form a well developed humus podzol with the B horizon at about 30—50 cm below the surface and the A_1, A_2, B_1, B_2 and C mineral horizons are well marked. The organic horizon is well developed varying from 10 to 15 cm in thickness. This is readily divisible into an L layer of the freshly fallen needles, an F layer in which signs of decomposition are apparent and an H layer of black humified material with no structure visible to the naked eye. The F layer can further be divided into F_1 and F_2 layers following KÚBIENA (1953). The microbiological activity of the profile as a whole is separable into two spheres, that in the organic horizons and that in the mineral soil. While there is clear evidence of the inter-dependence of the two spheres, they do, however, differ in many respects.

A detailed account of the L and F layers has been given recently (KENDRICK and BURGES, 1962) therefore only a general account of the microbiology of these layers will be given here. Fungal infection of the needles begins at least 5—6 months before the needles die and fall. The main needle fall is in August with a secondary fall in May. *Coniosporium*, *Lophodermium*, *Fusicoccum* and *Pullularia* are well established before the needles reach the ground and join the L layer. The active parasite *Coniosporium* disappears rapidly after the needles reach the ground but *Pullularia* and *Fusicoccum* increase considerably. After about 3 months the *Pullularia* gradually declines but *Fusicoccum* and *Lophodermium* remain in an active vegetative condition until the late autumn and winter when both fungi sporulate vigorously and then die away.

The first wave of infection by the true soil fungi occurs soon after the needles reach the ground. *Desmazierella*, *Sympodiella*, *Helicoma*, *Trichoderma* and *Penicillium* gradually colonise both the surface and the interior of the needles. These remain in the L layer for about 6 months by which time they have become covered with more recently fallen needles and pass into the F_1 layer. The active invasion of the needles by the various fungi is reflected in the high oxygen uptake (2366 μl/g/5 hrs) recorded by PARKINSON and COUPS (p. 170). As more needles fall they compress the older needles and the microenvironment becomes much moister. This is accompanied by the sporulation of *Desmazierella*, *Sympodiella* and *Helicoma* in the late winter and early spring. Often the needles become densely covered with the conidiophores of these three fungi. At times up to 1,000 conidiophores of *Desmazierella* or 10,000 conidiophores of *Sympodiella* were recorded on a single needle. In the autumn some 13—15 months after the main needle fall the grazing activities of the meiofauna gradually remove most, if not all, of the conidiophores and associated spores. Many of the faecal pellets of the mites at this time show short lengths of empty hyphae, portions of conidiophores and spores. The needles stay in the F_1 layer for approximately two years and a second crop of conidiophores is formed in the following spring. These in turn are grazed, mainly by mites.

Basidiomycete mycelium begins to make its appearance some 5 to 6 months after the needles enter the F_2 layer. Gradually the amount of basidiomycetes increases and recent studies indicate that they play a major role in the decomposition at this phase. The most abundant fruiting species in the litter are *Marasmius androsaceus*, *Lactarius rufus* and *Hygrophoropsis aurantiaca* but is has not yet been possible to correlate the mycelium seen in the needles with any one of these species. *Penicillium* and *Trichoderma* are also abundant in the F_2 layer. The length of time that the needle spends in the F_2 layer is difficult to estimate. It is at least 7—8 years before the needle is completely disintegrated. Fungi do not carry out the whole of the decomposition. Despite the abundance of fungi many needles are only partly invaded and histological examination shows that some quite extensive areas of tissue are virtually devoid of fungal hyphae and are converted directly by endophages to faecal pellets.

The meiofauna recorded from the litter included:

Enchytraeidae	*Friderica* spp.
Oribatidae	*Adoristes ovatus*
	Carabodes minusculus
	Ceratoppia bipilis
	Chamobates incisus
	Odontocepheus elongatus
	Oppia unicarinata
	Pelops tardus
	Platynothrus peltifer
	Thyrisoma lanceolata
Collembola	*Orchesella cincta*
	Lepidocyrtus sp.

In addition to the falling needles there is also a steady accumulation of branches, cones and bract scales on to the forest floor. No attempt has been made as yet to study the microbiological changes associated with their decay. They do nevertheless represent about 15% of the total litter which falls and often form specialized habitats for certain species. *Hydnum auriscalpium* for instance seems restricted to fallen cones.

With the continued activity of the fauna and microflora the needles are gradually reduced; carbon is lost as carbon dioxide and soluble materials are leached away and eventually the residues accumulate at the surface of the mineral soil to form the H layer. Microscopic investigation shows that this is far from being amorphous. It appears to be formed largely by the accumulation of faecal pellets together with exoskeletons from the meiofauna. The faecal pellets, although relatively firm when fresh, gradually disintegrate releasing the partially digested hyphal and cell fragments. In the dispersed debris fungal spores are frequent. Isolation techniques have shown that *Penicillium*, *Trichoderma* and *Mortierella* are widespread in the H layer, *Mortierella* spp. often predominating. The abundance of chitinous material in this layer suggested that some of these organisms might be associated with chitin breakdown and this has been confirmed by GRAY and BELL (see p. 227). The fungi in this layer have been investigated by GAMS (see p. 226 and p. 176). It would seem that these fungi are concerned with the final decomposition of residues from the overlaying material. Some species, e.g. *Mortierella paruispora*, *Stachybotrys* and *Trichoderma* are almost certainly chitin splitters, others seem to lack this ability and may be associated with the breaking down of cutin and other residues.

The ultimate fate of the residues in the H layer is partly complete mineralization and partly to be washed down into the mineral soil.

When we come to examine the microbiology of the mineral soil some of the problems are very different. In the litter a needle may be selected and the substrate for the organism be clearly defined. The well marked periodic fall of needles leads to a fairly readily recognizable succession in which the time sequence in the litter is represented as a spatial layering. In the mineral horizons it is often impossible to relate the fungal hyphae to a readily separable substrate. Although fine roots are shed annually and successions undoubtedly occur, they will be very difficult to demonstrate. For these reasons we have, in the first instance, been forced to rely on species lists and total activity measurements.

The isolation and identification of fungi from soil has been the subject of many discussions (e.g. WARCUP, 1960) and it has now become well accepted that most isolation methods bias the species lists very heavily in favour of actively sporing species. For this reason we have applied the washing technique developed by Parkinson and Williams to the mineral horizons. An account of this is given by WILLIAMS (p. 158). It will be seen from his work that the lists contain many of the generally accepted soil fungi. The washing technique has, however, enabled him to show that while some fungi seem to be present mainly as spores, others appear to occur as hyphae closely associated with organic fragments or large mineral grains and sporulate only sparsely or not at all.

Concurrently with the isolation work of Williams and the respiratory measurements undertaken by PARKINSON and COUPS (p. 170) we have attempted to study the fungi in situ, particularly by means of soil sections. This has been done primarily by Mr. Nicholas and had several objects in view. First we wished to see if we could obtain a measure of the amount of hyphae present in a given volume of soil in each of the different horizons. This has met with some success (BURGES and NICHOLAS, 1961) and we have obtained a considerable amount of information regarding the relative amounts of hyphae in the different horizons. Subsequent work suggests that the measurements obtained are underestimates and that the true amounts of hyphae may be 5 to 6 times the amounts we have been recording. It would seem that if a simple measure of length of hyphae is required the JONES and MOLLISON (1948) technique is considerably superior. Second we wished to gather information on the detailed relationships between the fungal hyphae and the soil particles. In this direction the soil sections have been highly successful. They have also yielded a considerable amount of information on the distribution of amorphous soil organic matter in relation to the soil cavities.

In the A_1 horizon the leached sand grains are loosely packed with large open soil cavities and the amorphous blackish organic matter gives the appearance of having been flocculated. Fungal hyphae are usually closely associated with the masses of organic matter and when passing from one mass of organic matter to another may travel relatively long distances without branching and are only deflected sufficiently to pass between the quartz grains. Hyphae of different types appear to intermingle and there is no evidence of any demarcation of territory by any one fungus.

In the A_2 horizon the distribution of the hyphae is somewhat similar but there is a tendency for some hyphae to be closely attached to sand grains. The deposition of iron oxide and humic materials in the B_1 horizon considerably alters the soil structure. In many places the soil cavities are completely occluded and the grains heavily coated. Most of the hyphae seen were closely associated with the soil particles and crossed from one grain to another at points of contact. Only occasional were hyphae seen to cross the soil cavities. Many of the hyphae seemed old and partially mummified by the encrusting humic acid. There is evidence to indicate that the alternate shrinking and swelling of the colloidal deposits causes a breaking of the older and presumably more brittle older hyphae. It is possible that the absence of suitable soil cavities prevents soil animals from attacking the hyphae and in the absence of any substantial bacterial population the hyphal walls remain long after they have ceased to function.

In the C horizon the total amount of hyphae is small and the hyphae tend to occur in aggregates in contrast to the more disperse distribution seen in the A horizons.

Table I shows the measurement of microbial activity by three very different methods. It will be seen that there is general agreement in some readings but each is measuring a different facet of the microbiological situation.

Reference has been made above to the marked seasonal and successional changes seen in the microflora of the organic horizon. Neither Dr. Williams with his washing technique, nor Dr. Parkinson and Miss Coups with their oxygen uptake measurements found obvious seasonal changes in activity in the mineral horizons. In both these studies soil moisture seemed to be the major factor determining fungal activity. On the other hand there is some indication from the results obtained by Mr. Nicholas of changes in the amount of fungal hyphae in the A_1 horizon and to a lesser extent in the A_2. Two peaks were recorded in the periodic samples; a larger following the main August leaf fall and a smaller shortly after the April-May leaf fall. It would seem that the influence of the needle fall does not penetrate beyond the A_2 horizon.

No reference has been made so far to bacterial or actinomycete action. Bacteria do occur in the profile but in low numbers, usually well below 10^5 per gram. Actinomycetes have been detected mainly in studies using the Jones and Mollison technique and marked variations in the amounts of actinomycete mycelium have been recorded, particularly in the B_1 horizon.

TABLE 1. Microbial activity measured by three different methods

Horizon or Layer	O_2 uptake/g	Dilution plate count	Metres of hyphae/ml soil sections
L	2366		
F_1	1400		
F_2	245		
H	81	281,900	5.56
A_1	13	199,800	3.96
A_2	4	27,250	3.78
B_1	10	11,370	1.09
B_2	3	1,970	0.37
C	1	225	0.03

Summary

The fungal growth and activity in the distinct layers of a podzol profile in a forest of *Pinus sylvestris* on glacial sand is described. The value of using different techniques, a.o. washing technique, soil sections, oxygen uptake measurements, in one investigation is demonstrated.

References

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Discussion

E. G. MULDER: What are the pH-values of the different layers?

A. BURGES: Ao as a whole has a pH of 4.5 to 5.5, the H may be as low as 2.8. Mineral horizons vary around pH 4.

M. OOSTENBRINK: Could you estimate what would have happened in your profile if no animals at all had been present to initiate the process of decay?

A. BURGES: I think the process would come to a standstill. Although some fungi can break down fungal walls, the conditions in the F layer are perhaps too dry for such action.

R. MANKAU: There was a conspicuous lack of the mention of Nematoda in your remarks. Is this due to their infrequency in the soil considered or the absence of techniques to determine their presence?

A. BURGES: The classification of nematodes from soil is exceedingly difficult and we did not employ techniques which would be necessary for the isolation and enumeration of this group. "They were conveniently overlooked".

T. R. G. GRAY: (Also replying to Dr. Mankau): We have buried chitin strips in some of these soils. The strips contain a large percentage of protein nitrogen which might be expected to stimulate nematodes. None the less they were only rarely observed. Neither have nematodes been seen on the plant roots.

W. KÜHNELT: Sie haben festgestellt, dasz die Milbenlosung, die im inneren, noch nicht von Mikroorganismen angegriffene Kiefernadeln abgesetzt wurde, gelbbraun gefärbt ist, während solche an von Mikroorganismen befallenen Nadeln dunkelbraun ist. Kann man bei letztere von einer ,,Humifizierung" sprechen?

A. BURGES: I believe that it is correct to regard this as the beginning of humification. I think "humic acids" arise by the oxidative polymerisation of phenol materials which may come from the plant debris or from the decomposition of substances such as lignin or from microbial synthesis.

A. JONGERIUS: It is possible to make a fairly exact measurement of the length of fungal hyphae in a certain horizon in the thin sections by the determination of the volume of the hyphae by aid of Zeiss integrating eye pieces. After measurement of the diameter of the hyphae one can calculate directly their total length.

A. BURGES: The difficulty is that in general one does not see all the fungi in the slides, especially not those in the dark organic matter.